REMARKS/ARGUMENTS

Before turning to the status of claims and the rejections, Applicants note the new Power of Attorney and Change of Address form filed with Applicants' prior response on February 9, 2006. The Examiner is respectfully requested to mail all future communications to the new address, to the attention of the undersigned attorney.

Claims 1-25 are pending in this application. Applicants note the withdrawal of the earlier rejection under 35 U.S.C. §112, first paragraph, for alleged failure to meet the written description requirement. It is noted that while the Office Action indicates that the rejection was withdrawn "in view of applicants' amendments dated 02/09/2006, this is clearly in error, since Applicants filed no claim amendments, rather addressed the rejection solely by legal arguments.

All claims remain rejected under 35 U.S.C. §103(a). The rejections are respectfully traversed, for reasons detailed below.

Claim Rejections - 35 U.S.C. §103

1. Claims 1-7, and 9-24 remain rejected as allegedly obvious over Hart *et al.* (BIO/TECHNOLOGY Vol. 12, November 1994) in view of Wetzel *et al.* (EP 0155189). Hart *et al.* was cited for its disclosure of a process for large-scale production of IGF-I from the periplasm of *E. coli* by culturing *E. coli* host cells having a plasmid comprising an inducible promoter and nucleic acid encoding a signal sequence for secretion into the periplasm linked to human IGF-I. In addressing Applicants' arguments submitted in response to the previous Office Action, the Examiner notes that the "claims as written do not exclude any type of *in situ* solubilization technique and aqueous two-phase extraction." Wetzel *et al.* was cited for its teaching of a plasmid comprising an inducible promoter and nucleic acid encoding a T4 phage lysozyme.

According to the rejection, it was "well known in the art that elimination or reduction of contaminating biological materials including proteoglycan and polysaccharide components of the bacterial cell is important for the heterologous expression and purification of a desired protein." Therefore, "one of ordinary skill in the art . . . at the time the invention was made would be motivated to eliminate or reduce proteoglycan and polysaccharide components of the *E. coli* bacterial cell wall such that the *E. coli* host cell taught by Hart et al. is further transformed with

the plasmid vector of Wetzel et al." The Examiner notes that "[e]limination or reduction of proteoglycan and polysaccharide components of the *E. coli* bacterial cell by action of the expressed lysozyme would enable a simpler purification of IGF-I or of any desired protein."

Applicants respectfully disagree, and vigorously traverse the rejection.

As discussed in Applicants' previous response, Hart *et al.* describe a 10-kiloliter-scale process for recovery of periplasmic IGF-I polypeptide from *E. coli*. High level expression of human IGF-I with a lamB signal sequence in *E. coli* led to accumulation of periplasmic refractile bodies (page 1113, second column). First, the authors attempted the typical isolation procedure involving a mechanical cell breakage step followed by a centrifugation step to recover the protein from the refractile bodies. The results were disappointing in that almost 40% of the total product was lost to the supernatant after three passes through the Gaulin homogenizer (see the results shown in Table 1 of the paper). In order to enhance recovery yield, a new procedure, including *in situ* solubilization by a chaotrope and a reductant, and two-phase extraction involving the addition of a polymer and salt to the solubilization mixture, was developed. At the end of the abstract, the authors state that together, "the techniques of *in situ* solubilization and aqueous two-phase extraction provide a new, high yield approach for isolating recombinant protein which is accumulated in more than one form during fermentation."

Wetzel *et al.* (EP 0 155 189) disclose a method to induce a recombinant cell culture to produce lysozymes without killing the bacterial host cells.

A prima facie case of obviousness has not been established.

Applicants maintain their earlier position that there is no sufficient motivation to combine Hart *et al.* and Wetzel *et al.* The requirement that an Examiner must show a suggestion to combine references cited in support of an obviousness rejection is a critical safeguard against hindsight reconstruction of an invention. The motivation to modify a reference can come from: (1) the nature of the problem to be solved, (2) the teachings of the prior art itself, or (3) the knowledge of persons of ordinary skill in the art. In re Rouffet, 149 F.3d at 1358; 47 U.S.P.Q.2d at 1458.

The nature of the problem to be solved by Hart *et al.* and Wetzel *et al.*, respectively, is significantly different. Hart *et al.* aim at providing an efficient, simplified large-scale process for the purification of IGF-I. Wetzel *et al.* produce lysozyme in a recombinant host cell without killing the host. Therefore, the motivation to combine does not come from the problem itself.

Nor does the motivation to combine come from the teachings of the cited prior art. There is absolutely no teaching or suggestion in Hart *et al.* that elimination or reduction of the proteoglycan components of the *E. coli* bacterial cell wall would have been a problem that Hart *et al.* faced and tried to address. Indeed, Hart *et al.* clearly provide an efficient method for recovery of a recombinant protein, IGF-I from the periplasmic space of *E. coli* without reliance on a lysozyme to lyse cell walls.

By stating that it was "well known in the art that elimination or reduction of contaminating biological materials including proteoglycan and polysaccharide components of the bacterial cell is important for the heterologous expression and purification of a desired protein" the Examiner appears to imply that the motivation to combine came from the knowledge of persons of ordinary skill in the art. However, this knowledge has not been established by any evidence, and even if it had, in the absence of any established need to apply this knowledge, a motivation to combine the two references cited would still not have existed.

Even if Hart et al. and Wetzel et al. could be properly combined, the combination would still not make obvious the invention claimed in the present application.

There is nothing in either Hart *et al.* or in Wetzel *et al.* suggesting that coexpression should be performed using certain types of promoters, and by inducing the expression of the phage lysozyme only after about 50% or more of the heterologous protein has accumulated, as required by the language of claim 1. Finally, there is no teaching or suggestion in either Hart *et al.* or Wetzel *et al.* to perform each step of the process in the absence of chloroform, also as required by the language of claim 1. Accordingly, even if Hart *et al.* and Wetzel *et al.* could be properly combined, the combination would still not result in the invention as claimed. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the present rejection.

2. Claim 8 was rejected under 35 U.S.C. §103(a) as "being unpatentable" over Hart *et al.* in view of Wetzel *et al.* "as applied to the claims above," and further in view of Wick *et al.*, Infect. Immun. 1993, Nov., 61(11):4848-56. Wick *et al.* was cited for its teaching of nucleic acid encoding the lamB signal sequence for expression in the periplasm of *E. coli*.

As explained in response to the previous rejection, the combination of Hart *et al.* and Wetzel *et al.* is improper and does not yield the claimed invention. Since Wick *et al.* does not cure the underlying discussed deficiencies of the primary references with respect to Claim 8, the present rejection should be withdrawn for the same reasons as the foregoing rejection of Claims 1-7 and 9-24.

3. Claim 25 was rejected under 35 U.S.C. §103(a) as "being unpatentable" over Hart *et al.* in view of Wetzel *et al.* "as applied to the claimed above," and further in view of Balbas *et al.* (Gene, 1996 June 12:172(1):65-9). Balbas *et al.* was cited for its teaching of the plasmid pBRINT, which is an efficient vector for chromosomal integration of clones DNA into the lacZ gene of *E. coli*, and of the method for such integration, and that the integration is advantageous with respect to stability or undesired copy number effects.

As explained in response to the previous rejection, the combination of Hart *et al.* and Wetzel *et al.* is improper and does not yield the claimed invention. Since Balbas *et al.* does not cure the discussed deficiencies of the primary references, the present rejection should be withdrawn for the same reasons as the foregoing rejection of Claims 1-7 and 9-24.

All claims pending in this application are believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any additional fees for extension of time, or credit overpayment to Deposit Account No. **08-1641** (Attorney's Docket No. **39766-0128 A**).

Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: September 5, 2006

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